International Journal of Zoology
and Research (IJZR)
ISSN(P): 2278-8816; ISSN(E): 2278-8824
Vol. 5, Issue 4, Aug 2015, 19-26

SCANNING ELECTRON MICROSCOPIC (SEM) AND ENERGY DISPERSIVE ANALYSIS OF X-RAYS (EDAX) STUDIES ON EGG SHELL OF SILKWORM, *PHILOSAMIA RICINI* (LEPIDOPTERA: SATURNIIDAE)

DEEPAK DEWAJI BARSAGADE[1], MINATAI VIJAY CHAMAT[2], IZHAR RAZA HUSAIN[3], HEMRAJ MADHUKAR MESHRAM[4] & RANI PRAMODRAO THAKRE[5]

[1,3,4,5]Department of Zoology, MJF Educational Campus, RTM Nagpur University, Nagpur Ms India

[2]Vittalrao Chamat High-School Dighori, Nagpur Ms India

ABSTRACT

Scanning electron microscopic studies on the egg shell of eri silkworm, *Philosamia ricini*, revealed the differentiation into two zones, the micropylar and disc or flattened zone. The micropylar zone is surrounded by rosette like cells. The various shaped follicular cell imprints (FCI) are scattered throughout the disc zones. A large aeropyle present at the centre of FCI while each junction of three follicular cell imprints contains small aeropyle. Each FCI are surrounded by six aeropyles. The centrally placed large aeropyle is deep and contain three to four opening. Quantitative analysis of elements by using the Energy Dispersive Analysis of X-rays (EDAX) techniques confirmed presence of elements on the egg shell. Total ten elements like carbon, oxygen, calcium, sulphur, chlorine, potassium, sodium, magnesium, silicon and phosphorous were detected on the egg shell surface. Six elements are common on FCI and space between FCI, while four elements are limited to FCI only. The differences between presence of elements on FCI and in between were discussed.

KEYWORDS: Philosamia Ricini, Egg Shell, SEM, EDAX

INTRODUCTION

The structure of insect egg shell are quite complex and consists of the vitelline envelop and chorion (Kumar *et al.,* 2002). The chorion is secreted by the follicle cells of vitellogenic oocytes and in many insects it is composed of two layers, the inner endochorion and outer exochorion. The chorion protects the oocyte from mechanical and environmental stresses and at the same time permits gas exchange and sperm penetration (Andrew and Tembhare, 1995, 1996; Belles *et al.,* 1993; Regier and Kafatos, 1985). In some species, a layer is formed below the endochorion as an inner most layer of crystalline chorion (Margaritis, 1985). The scanning electron microscope has very greatly advanced the knowledge of the overall architecture of insect egg shells bringing with a better understanding of the respiratory process (Hinton, 1970). The morphology and architectural pattern of the chorion in different insects depends on the imprints of the follicular secretory cells on specialized regions such as micropyles, aeropyles and flat regions (Beament, 1948 and Regier *et al.,* 1980). The outermost layer of endochorion is characterized by the presence of lamellae based on the hexiocoidal arrangement of stacks of fibrils. The exochorion consists of mucoproteins and polysaccharides, and this may form house channels to permit entry of sperms, aeropyles for gas exchange and lines of weakness to facilitate hatching (Hinton, 1969). The addition of an X-ray analyzer to the scanning microscope pursuits the measurements of the local concentration of the elements phosphorus and sulphur which help greatly in the characterization of structural components (Furneaux and Mackay, 1976). The elemental composition of some invertebrate eggs differs between species and populations and is correlated with habitat features

(Baur and Baur, 1998; Anger *et al.,* 2002). In butterflies (Lepidoptera) the amount of nitrogen content required to build the eggs is largely fixed before adult eclosion, while nitrogen compound are essential for vitellogenesis (Rivero and Casas, 1999; Shapiro & Ferkovich, 2002; Wall *et al.,* 2002). Most of the nitrogen to be allocated to the eggs has to be obtained by the larval stages while the carbon derived elements come from both, the larval and adult diets (Boggs & Ross, 1993; O'Brien *et al.,* 2004).

Very little information is available about the elemental composition of insect eggs except initial work of Nickles *et al.,* (1995), Scewczuk *et al.,* (2010) and Sreedevi *et al*., (2014).

Therefore, the present SEM and EDAX studies is carried out to know the egg shell ultra structure and elemental composition of egg chorion in eri silkworm, *P. ricini*.

MATERIALS AND METHODS

The eggs of eri silkworm, *P. ricini* were examined by scanning microscopy. The freshly laid eggs were washed thoroughly with distilled water and fixed in 10% formalin for a period of 12 h, dehydrated in various grades of alcohol, cleared in acetone, dried and fixed on the metallic stub precoated with carbon strip at different angles. Eggs fixed on stub were processed for platinum coating and scanned under the Jeol (JSM 6380A) Scanning Electron Microscope (SEM) and Energy Dispersive Analysis of X-rays (EDAX) at Visvesvaraya National Institute of Technology (VNIT), Nagpur, India.

RESULTS

The surface of the egg is differentiated into two zones, a) The micropylar zone and b) The disc or flattened zone (Figure. 1).

The Micropylar Zone

The micropylar region is a deeply concealed area forming a distinct micropylar pit of about 5 ± 2 µm in diameter at the anterior pole of anterioposterior axis. There are 7–8 micropyles in the micropylar pit and are widely separated from each other. Each micropyle is about 0.8 to 1.2 µm in diameter. The micropylar area is surrounded by a group of radiating petaloid cells. The petaloid cells show narrow proximal margin towards the micropylar area whereas the distal margin is wider and rounded forming a sort of rosette around the micropylar pit. In the group of radiating petaloid cells primary micropylar cells are surrounded by secondary and tertiary layers. The petaloid cells of primary to quaternary layers change in size gradually and become hexagonal in shape (Figure. 2, 3).

The Disc Zone

The disc or flattened zone occupies major surface of the egg. The FCI are joined to each other with less intercellular space. The FCI are hexagonal, small as well as large in shape. The aeropyles are situated at the joints of FCI and widely separated from each other and do not form pair. The numbers of aeropyles are various ranging from 5 to 7. At the centre of each FCI a big aeropyle is present with four to five opening (Figure. 4, 5).

Energy Dispersive Analysis of X–rays (EDAX)

With the help of Energy Dispersive Analysis of X–rays (EDAX) techniques the quantitative analysis of elements present on the egg shell of *Philosamia ricini* was observed. Total ten elements were detected on the different zones of egg shell surface.

On the micropylar zone in the follicular cell impression (FCI) five elements are detected, *viz.* Carbon, Oxygen, Sulphur, Chlorine and Potassium and their mass percentage are 79.56%, 9.11%, 4.03%, 3.94% and 3.36% respectively (Figure. 6).

The intercellular space of micropylar cells (FCI) contains Carbon 62.46%, Oxygen 22.40% and Sulphur 4.32% respectively (Table 1).

On the disc zone eight elements are detected on the FCI and they are Carbon, Oxygen, Calcium, Potassium, Sodium, Magnesium, Silicon and Phosphorus (Figure 7), while only four elements i.e., Carbon Oxygen Sulphur and Chlorine was detected in between the aeropyles of inter cellular space of edge zone FCI.

The disc zone of egg shell shows the presence of Carbon 36.39%, Oxygen 26.75%, Calcium 22.12%, Chlorine 2.05% and Phosphorus 8.79%, on the FCI (Table 1), while Carbon, Oxygen, Calcium, Sulphur, Chlorine, Potassium and Sodium are detected from the area in between aeropyles of inter cellular disc zone FCI.

DISCUSSIONS

The surface ultrastructure of the egg chorion of *P. ricini* reveals differentiation of the entire egg surface into micropylar and disc zones. The edge margin is in-distinct and marginal follicular cell imprints (FCI) are distinct than the FCI of disc zone. Differentiation of the chorionic surface into four zones has also been reported in *A. polyphemus* and *A. pernyi* (Kafatos *et al.,* 1977; Mazur *et al.,* 1982), whereas in *A. mylitta* edge zone was prominent (Barsagade *et al.,* 2009). However the area of these zones and the size of the aeropyles vary from species to species. The chorion thickness was about 50 µm and 60 µm thick in *A. polyphemus* and *A. penyi,* respectively (Margaritis, 1985). Thick chorion was also found in *Samia ricini* (Kumar *et al.,* 2007). The thick chorionic egg shell of the silk moth is advantageous from environmental protection, predation and parasitization point of view.

In *P. ricini,* the micropyles are present at the margin of the micropylar pit which is surrounded by a rosette of petaloid cells similar to that reported by Kumar *et al.,* (2007) in *Samia ricini, A. mylitta* (Barsagade *et al*., 2009) and tasar silkworm and other species of Lepidoptera (Margaritis, 1985).

All micropyles penetrate through the chorion and vitelline membrane by canals which radiate outwards from the micropylar opening and traverse the chorion at an angle of 45^0 (Margaritis, 1985, Kumar *et al.,* 2007). In the present study it has been observed that the micropyles penetrate the egg shell chorion and vitelline membrane internally. The present observations confirmed the earlier studies. The aeropyles are present in the form of aeropylar crowns in the area of egg shell edge zone reported in Saturniid silkmoths, *A. mylitta* (Barsagade *et al*., 2009). The posterior surface of egg shell with or without shell imprints contain the aeropyles in a pit (sunken aeropyles) while FCI of micropylar zone contain aeropyle in egg of *S. oblique* (Kumar *et al.,* 1999) and *Spodoptera littoralis* (Fehrenbach *et al., 1*987). In *P. ricini* the aeropyles are present on the FCI of micropylar zone. In *P. ricini* two types of aeropyles are present on the FCI. The large aeropyles situated at the centre of FCI with many opening and small aeropyles on junction of FCI. The presence of centrally placed aeropyles with an opening might be the species specific characters of *P. ricini.* In insect eggs aeropyles covered more than 12% of the surface and correspond to the plastron surface of about 4 x 105 mm2/mg egg weight (Hinton, 1969). The presence of similar large chorion surface in *P. ricini* may correspond to the terrestrial plastron respiration. The aeropyles found at the three cells imprint junction in *P. ricini* are similar to that reported in *A. polyphemus* (Margaritis, 1985).

Energy Dispersive Analysis of X–rays (EDAX) has confirmed the presence of ten elements on the egg shell of *Philosamia ricini.* The detected elements are Carbon, Oxygen, Calcium, Sulphur, Chlorine, Potassium, Sodium, Magnesium, Silicon and Phosphorus. The 6 elements viz., Carbon, Oxygen, Calcium, Sulphur, Chlorine and Potassium are common on FCI and on the junction of FCI while, Sodium, Magnesium, Silicon and Phosphorus are limited on FCI. Earlier Oxygen, Carbon, Nitrogen, Phosphorus, Sodium, Sulphur, Magnesium, Calcium, Silicon, Chlorine and Potassium were detected in the eggshell on different location (Nickles *et al.,* 1995; Nickles *et al.,* 2002). The elements like Carbon, Nitrogen, Oxygen, Magnesium, Phosphorus, Sulphur, Calcium and Zinc were reported in the eggs of honey bee, *Apis mellifera* (Abou-Shaara *et al.,* 2013). The presence of magnesium, calcium and Phosphorus are associated to each other during biochemical processes (McDonald *et al.,* 1971). According to Attech *et al.,* (1982) sufficient level of Magnesium and Calcium together improves the egg shell strength. The presence of sufficient levels of Magnesium along with Calcium indicating good strength of egg shell of *P. ricini.* The carbon derived element come from both, the larval and adult diets (Boggs, 1997; O'Brien *et al.,* 2004). Since the adults are non feeders in *P. ricini* the carbon derived element possibly came from larval diet. In majority of terrestrial insect eggs, different types of chorionic respiratory system evolved with meshwork in the chorion that holds a layer of gas and supply oxygen to egg (Hinton, 1969). The presence of Carbon and Oxygen levels in Scarabaidae egg chorion is related to the respiratory function of eggs (Sreedevi *et al.,* 2014). In the present investigation it has been observed that the Carbon and Oxygen are present in sufficient level indicating eggshell is involved in respiratory function.

CONCLUSIONS

According to Sreedevi *et al.,* (2014) differences and similarities in elemental composition and proportion are attributed to the egg properties and chorionic ultra structures. The present study revealed that the total ten elements are found on the different parts of eggshell in *P. ricini* might be playing important role during the formation of multilayered complex structure of chorion.

REFERENCES

1. **Abou-shaara, H. F., Ahmad, A., Al-ghamdi., & Abdelsalam, A. M. (2013).** Elemental analysis of eggs for two honey bee races. Ira J Entomol, **3,** 14-17.

2. **Andrew, R. J., &Tembhare, D. B. (1995).** Ultrastructural post-oviposition changes in the egg chorion of the dragonfly *Zyxomma petiolatum,* (Rambur) (Odonata: Libellulidae). Int J Insect Morphol and Embryol, **24,** 235-238.

3. **Andrew, R. J., & Tembhare, D. B. (1996).** Surface ultrastructure of the egg chorion of *Bradinopyga geminata* (Rambur) and *Rhyothemis variegate variegate* (Linn.) (Anisoptera: Libellulidae) Fraseria (N. S.), **3,** 1-5.

4. **Anger, K., Moreira, G. S., & Ismael, D. (2002).** Comparative size, biomass, elemental composition (C, N, H), and energy concentration of caridean shrimp-eggs. Invert Reprod Dev, **42,** 83–93.

5. **Atteh, J. O., & Leeson, S. (1982).** Influence of increasing dietary calcium and magnesium levels on performance, mineral metabolism and egg mineral content of laying hens. Poult Sci, **62,** 1261-1268.

6. **Barsagade, D. D., Pankule, S. D., & Tembhare, D. B. (2009).** Impact of fungus on egg shell of tropical tasar silkworm, *Antheraea mylitta*: An ultra- structural Approach. Int J Indust Entomol, **18(2),** 77-82.

7. **Baur, A., & Baur, B. (1998).** Altitudinal variation in size and composition of eggs in the land snail Arianta arbustorum. Can J Zool, **76,** 2067–2074.

8. **Beament, J. W. L. (1948).** The penetration of insect eggshell. I. Penetration of the chorion of *Rhodnius prolixus.* Stal Bull Entomol Res, **39,** 359-383.

9. **Bells, X. P., Cassier, S. C., Pascual, N., Andrew, M., Rosso, Y., & Piulachs, M. D. (1993).** Induction of choriogenesis by 20-hydroxy-ecdysone in the German cockroach. Tissue cell, **25,** 195-206.

10. **Boggs, C. L., & Ross, C. L. (1993).** The effect of adult food limitation on the life history traits in Speyeria mormonia (Lepidoptera: Nymphalidae). Ecology, **74,** 433–441.

11. **Boggs, C. L. (1997).** Dynamics of reproductive allocation from juvenile and adult feeding: radiotracer studies. Ecology, **78,** 192–202.

12. **Furneaux, P. J. S., & Mackay, A. L. (1976).** The composition, structure and formation of the chorion and the vitelline membrane of insect egg shell. *In* The insect integument (Ed. H. R. Hepburn) Published by Elsevier Scientific Publishing Company.

13. **Hinton, H. E. (1969).** Respiratory systems of insect egg shells. Annual Review of Entomology, **14,** 343-368.

14. **Hinton, H. E. (1970).** Insect egg shell. Sci Am, **223,** 84-91.

15. **Kafatos, F. C., Regier, J. C., Mazur, G. D., Nadel, M. R., & Balu, H. M. (1977).** The Eggshell of Insects: Differentiation-specific Proteins and the Control of Their Synthesis and Accumulation During Envelopment. In Results and Problems in Cell Differentiation (Eds by Beeman, W.) Springer Verlag Berling, **8,** 45-145.

16. **Kumar, V., Kariappa, B. K., Babu, A. M., &Dandin, S. B. (2007).** Surface ultrastructure of the egg chorion of eri silkworm, *Samia ricini* (Donovan) (Lepidoptera: Saturniidae). J Entomol, **4,** 68-81.

17. **Margaritis, L. H. (1985).** Structure and Physiology of the Eggshell. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology (Eds by Gilbert), Pergamon Press, Oxford 153-230.

18. **Mazur, G. D., Regier, J. C., & Kafatos, F. C. (1982).** Order and defects in the silkmoth chorion, a biological analogue of a cholesteric liquid crystal. In Insect Ultrastructure (Eds by Akai H. and King, R. C.) Plenum Press, New York.

19. **Mcdonals, P., Edwards, R. A., & Greenhalgh, J. F. D. (1971).** Animal Nutrition: 3rd Edition, London: Oliver and Boyd, 83-84.

20. **Nickles, E. P., Bakhru, H., Ghiradella, H. T., & Haberl, A. (1995).** Elemental analysis of the eggshell of the Karner Blue butterfly (*Lycaeides melissa samuelis)* using a nuclear microprobe. Nuclear Instruments and Methods in Physics Research B, **99,** 387-389.

21. **Nickles, E. P., Ghiradella, H. T., Bakhru, H., & Haberl, A. (2002).** Egg of the Karner blue butterfly (*Lycaeides Melissa samuelis*): morphology and elemental analysis. J Morphol, **25,** 140-148.

22. **O'brien, D. M., Boggs, C. L., Fogel, M. L. (2004).** Making eggs from nectar: connections between butterfly life history and the importance of nectar carbon in reproduction. Oikos, **105,** 279–291.

23. **Regier, J. C., Mazur, G. D., & Kafatos, F. C. (1980).** The silkmoth chorion: morphological and biochemical characterization of four surface regions. Dev Biol **76,** 286-304.

24. **Regier, J. C., & Kafatos, F. C. (1985).** Molecular aspects of chorion formation. In Comprehensive Insect Physiology, Biochemistry and Pharmocology (Eds by Kerkut, G. A. and Gilbert, L. I.) Pergamon Press, Oxford.

25. **Rivero, A., & Casas, J. (1999).** Rate of nutrient allocation to egg production in a parasitic wasp. Proc R Soc Lond. (B), **266,** 1169–1174.

26. **Shapiro, J. P., & Ferkovich, S. M. (2002).** Yolk protein immunoassays (YP-ELISA) to assess diet and reproductive quality of mass-reared *Orius insidiosus* (Heteroptera: Anthocoridae). J. Econ Entomol, **95,** 927–935.

27. **Sreedevi, K., Tyagi, S., & Rita devi, S. (2014).** Elemental analysis of egg chorion of white grubs belonging to Melolonthinae and Rutelinae (Coleoptera: Scarabaeidae), Current Biotica **8(1),** 42-48.

28. **Szewczuk, Z., Stefanowicz, P., & Nawrot, J. (2010).** The chemical composition of egg plugs deposited by *Sitophilus granaries* L. females on grain. *Proceedings of the 10th International Working Conference on Stored Product Protection, 27 June-2 July 2010, Estoril Congress Center, Portugal* (ed by Carvalho MO, Fields PG, Adler CS, Arthur FH, Athanassiou CG, Campbell JF, Fleurat-Lessard F, Flinn PW, Hodges RJ, Isikber AA, Navarro S, Noyes RT, Riudavets J, Sinha KK, Thorpe GR, Timlick BH, Trematerra P, White NDG), Julius Kühn-Institut, Berlin, 161-166.

29. **Wall, R., Wearmouth, V. J., & Smith, K. E. (2002).** Reproductive allocation by the blow fly Lucilia sericata in response to protein limitation. Physiol Entomol, **27,** 267–274.

APPENDICES

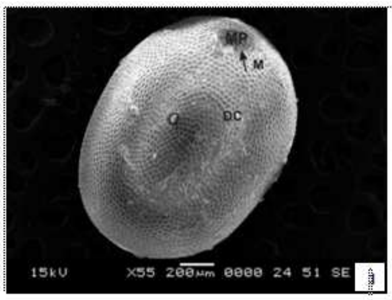

Figure 1: Scanning Electron Microscopy (SEM) Photomicrograph Showing the Surface Ultrastructure and Micropylar Pit (MP), at Anterior End

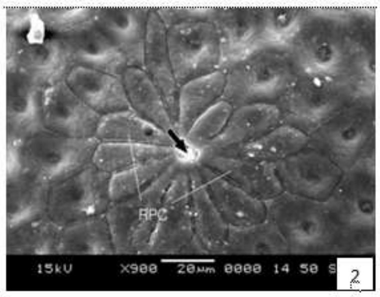

Figure 2: SEM Photomicrograph Showing the Micropylar Pit (Arrow), Surrounded by Rosette Petaliod Cells (RPC)

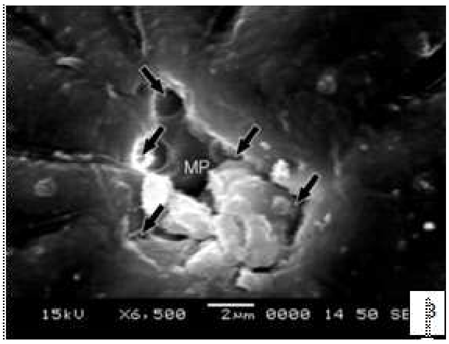

Figure 3: SEM Photomicrograph of Micropylar Pit Showing Micropyles (Arrow)

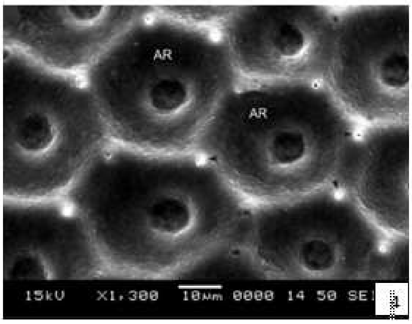

Figure 4: SEM Photomicrograph of Disc Zone Showing the Hexagonal FCI With Centrally Placed Big Aeropyles and Marginally Arranged Inter Cellular Small Six Aeropyles (Astric)

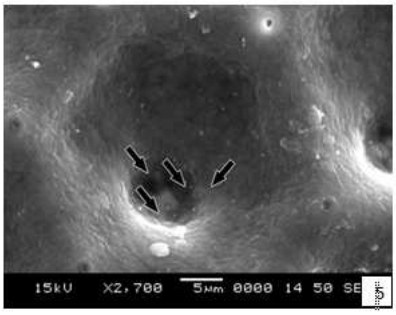

Figure 5: SEM Photomicrograph of Centrally Placed Big Aeropyles Showing Many Opening (Arrow)

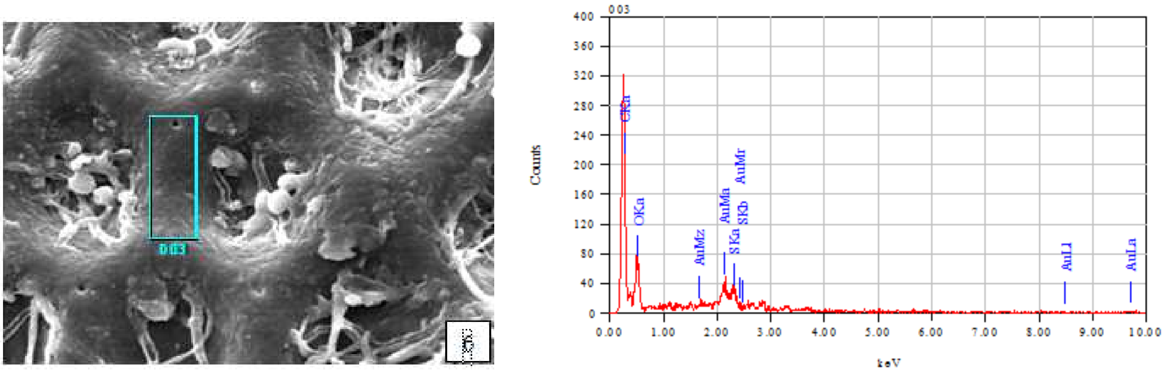

Figure 6: EDAX Micropylar FCI Zone Intercellular Space Showing Presence of Elements

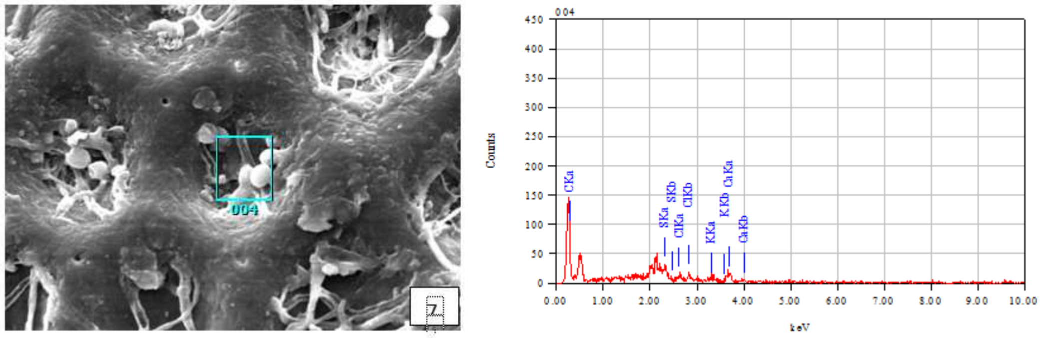

Figure 7: EDAX Disc Zone Intercellular Space Showing Presence of Element

Table 1: EDAX of Egg Shell of *P. Ricini* Showing Various Elements

Sr No	Element Present	Area of Egg Shell							
		Micropylar FCI Aeropyle	Mass % of Element	Interspace Area of Micropylar FCI	Mass % of Element	Disc Zone FCI Aeropyle	Mass % of Element	Interspace Area of Disc Zone FCI	Mass % of Element
1	Carbon	+	62.46	+	65.55	+	79.56	+	48.20
2	Oxygen	+	22.40	+	30.39	-	-	+	31.44
3	Calcium	-	-	-	-	+	8.92	-	-
4	Sulphur	+	4.32	+	4.03	-	-	+	2.75
5	Chlorine	+	1.32	-	-	-	-	+	3.94
6	Potassium	-	-	-	-	+	2.94	-	-
7	Sodium	-	-	-	-	+	1.23	-	-
8	Magnesium	-	-	-	-	+	1.19	-	-
9	Silicon	-	-	-	-	+	0.92	-	-
10	Phosphorus	-	-	-	-	+	5.16	-	-